From tree tops to the ground: Reversals to terrestrial habit in *Galeandra* orchids (Epidendroideae: Catasetinae)

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ABSTRACT

The colonization of the epiphytic niche of Neotropical forest canopies played an important role in orchid's extraordinary diversification, with rare reversions to the terrestrial habit. To understand the evolutionary context of those reversals, we investigated the diversification of *Galeandra*, a Neotropical orchid genus which includes epiphytic and terrestrial species. We hypothesized that reversion to the terrestrial habit accompanied the expansion of savannas. To test this hypothesis we generated a comprehensive time-calibrated phylogeny and employed comparative methods. We found that *Galeandra* originated towards the end of the Miocene in Amazonia. The terrestrial clade originated synchronously with the rise of dry vegetation biomes in the last 5 million years, suggesting that aridification dramatically impacted plant diversification and habits in the Neotropics. Shifts in habit impacted floral spur lengths and geographic range size, but not climatic niche. The longer spurs and narrower ranges characterize epiphytic species, which probably adapted to specialized long-tongued Euglossini bee pollinators inhabiting forested habits. The terrestrial species present variable floral spurs and wider distribution ranges, with evidence of self-pollination, suggesting the loss of specialized pollination system and concomitant range expansion. Our study highlights how climate change impacted habit evolution and associated traits such as mutualistic interactions with pollinators.

1. Introduction

The shift form a terrestrial mode of life to the epiphytic niche and the associated colonization of tropical forest canopies extraordinarily changed the diversification of orchids in Neotropics (Benzing, 1987; Gentry and Dodson, 1987; Chomicki et al., 2015a; Givnish et al., 2015). Epiphyte microhabitat specialization together with biotic and abiotic variables including pollinator shifts, CAM photosynthesis, and occurrence within cordilleras have been proposed as the main drivers of orchid diversification (Givnish et al., 2015; Pérez-Escobar et al., 2017a). The origin of epiphytism in land plants, including orchids, ferns, and leafy liverworts, followed the establishment of angiosperm-dominated canopies in the Cenozoic, which was possibly facilitated by climate change in the Paleocene/Eocene border (Chomicki et al., 2015a; Feldberg et al., 2014; Schuettpelz and Pryer, 2009)

Orchids are the most diverse group of vascular epiphytes, accounting for 68% (19,000) of the 27,600 species (Gentry and Dodson, 1987; Zotz, 2013). The evolution of epiphytism may have enhanced orchid diversification by the ecological opportunity offered by the conquest of new, largely unoccupied canopy niches (Givnish et al., 2015), and often terrestrial orchid lineages are less diverse than their epiphytic counterparts (Gravendeel et al., 2004). The epiphytic habit also offers the option of colonizing large and heterogeneous habitats: all the surface of branches, twigs and bark, which vary in light intensity, temperature level, bark structure and chemistry and more (Laube and Zotz, 2003). However it requires adaptation to low substrate stability, limited nutrient and water supplies, and habitat patchiness (Laube and Zotz, 2003). Thus, the canopy is difficult to colonize and only a plant with a complex suite of adaptations can survive as an epiphyte (Benzing, 1987). Orchid adaptations to tree bark includes root with

layer(s) of dead cells known as velamen, which enhance water and nutrient absorption and protects photosynthetic roots against UV-B radiation (Chomicki et al., 2015a), and thick succulent leaves and stems that store water (Freudenstein and Chase, 2015).

In Orchidaceae, epiphytism evolved at least four to seven times over the past 43 million years and was possibly lost about seven to ten times (Chomicki et al., 2015a). In the species-richest subfamily Epidendroideae the epiphytic habit predominates, yet the ancestral condition in orchid is clearly terrestrial (Chomicki et al., 2015a; Freudenstein and Chase, 2015). Reversals to the terrestrial habit occurred many times in Epidendroideae, including the Collabieae (Xiang et al., 2014), *Dendrobium* (Xiang et al., 2016), most *Eulophia* (Martos et al., 2014), *Cyanaeorchis* (Batista et al., 2014), *Bletia + Hexalectris + Basiphyllaea* (Sosa et al., 2016), a few Pleurothallidinae (Freudenstein and Chase, 2015), Malaxideae (Cameron, 2005) and in *Galeandra* (Catasetinae, *this study*). Although most reversals are associated to species-poor lineages, some are associated to speciose clades, potentially resulting from rapid diversification (Cameron, 2005). The ecological context driving reversal to the terrestrial habit is still poorly understood. Such reversals might also entail deep changes in morphological adaptations (Zhang et al., 2017) such as the loss or reduction of the velamen (Chomicki et al., 2015a) or biome shift (Sosa et al., 2016). Some of these morpho-anatomical adaptations are possibly linked to the presence of the *AGL12* gene, which regulates cell differentiation in underground roots and is otherwise lost in epiphytic orchids (Zhang et al., 2017).

To better understand the factors that might have influenced the reversal to the terrestrial habits in a linage within the predominantly epiphytic Epidendroideae, we investigate *Galeandra* diversification dynamics, climatic preferences, flower morphology and area of occurrence, using phylogenetic comparative methods. *Galeandra* is a widely distributed genus in the Neotropical region, ranging from southern Florida to northern Argentina, and five of its *ca* 20 species are terrestrial (Monteiro et al., 2010). *Galeandra* species occur across a wide range of biogeographic regions, mainly Amazonia, Cerrado savannas and the Atlantic Forest. Epiphytic *Galeandra* usually occupy more restricted distribution ranges than terrestrial species, and occurs in forested areas (e.g. Amazonia), while the terrestrial species occupy open vegetation ecosystems (e.g. Cerrado, except *G. beyrichii* which inhabits forest areas, especially altitude forests). The restricted distribution of epiphytic species in forests could be associated to habitat availability, specialist pollinator distribution or seed dispersal restriction due to the patchiness of tropical forests populations (McCornick and Jacquemyn, 2014, Winkler et al., 2009).

The origin of the savannas worldwide, including the south American Cerrado, coincides with the gradual cooling that started at the Miocene climatic optimum [~ 15 million years (Ma) (Zachos et al., 2001)], and they fully established about 5 Ma (Simon et al., 2009). Terrestrial *Galeandra* might have occupied these novel environments only recently. This raises the question whether terrestrial *Galeandra* are adapted to cooler and dryer habitats than epiphytic species occurring in rainforests. Furthermore, it is not clear whether the origin of terrestrial *Galeandra*, and potential biome shifts (from forested to open savannas) was triggered by the origin of the Cerrado savanna, an hypothesis that can be evaluated using a time-calibrated phylogeny and biogeographic analysis.

Habitat preference might also have driven the evolution of particular pollination syndromes, in particular because terrestrial and epiphytic *Galeandra* present different floral spur length and morphology. Floral spur enlargement is usually linked to pollinators shifts, and it has been hypothesized to be an adaptive response to predefined pollinator morphology (Whittall and Hodges, 2007). Flowers of epiphytic *Galeandra* have a long and filiform spur, while terrestrial taxa exhibit flowers with short and saclike spurs (Monteiro et al., 2010). Long floral spurs usually produce nectar or oil, and are accessed by an enlarged part of pollinator's body (e.g. tongues), but also legs (Steiner and Whitehead, 1990; Whittall and Hodges, 2007). However the extent to which habitat and different floral morphologies are correlated is largely unknown.

To investigate *Galeandra* diversification in time and space, and potential associated floral trait shifts, we used DNA sequences from Monteiro et al. (2010) plus newly generated sequence data of *Galeandra*, including a dense outgroup sampling. Our aim was to investigate when and where did *Galeandra* originate and when the terrestrial habit shift(s) occurred, in order to understand the ecological context of the reversal to the terrestrial habit. We hypothesized that habitat shifts accompanied the expansion of open vegetation areas during the Miocene. We also posited that terrestrial species would have larger range size than their epiphytic counterparts, possibly affecting leading to niche differentiation. Finally, we hypothesized – based on the premise that long floral spurs are associated to the long-tongued Euglossini bees (which are rare in savannas) – that terrestrial species would have a convergent pattern of floral spur length, possibly associated to other groups of pollinators.

2. Material and methods

2.1. Phylogenetics and dating analysis

To investigate the time of origin and diversification of *Galeandra* species we relied on a molecular dating approach. Our study builds upon the sampling of Monteiro et al. (2010) for *Galeandra* plus newly generated sequences for *G. leptoceras* and *G. macroplectra*, totalizing 85% (17 out of 20) of all species in the genus. The outgroup sampling was also enlarged to better accommodate molecular dating calibrations (see below), comprising representatives of all Catasetinae genera, including *Cyrtopodium* and *Eulophia,* and newly generated sequences of *Catasetum, Clowesia, Cyanaeorchis* and *Cycnoches*. Voucher information and GenBank numbers are presented in Table S1. DNA extraction, PCR conditions and sequencing methods are described in Monteiro et al. (2010).

The final matrix consisted of 31 taxa and 6014 nucleotides for five plastid (*ycf1, psbA-trn*H, *rpo*B-*trn*C and *trn*S-*trn*G), and three nuclear (*xdh*, ITS and ETS) markers. Alignments were performed in MAFFT v. 7 (Katoh and Standley, 2013), with default parameters except for the protein-coding markers were aligned using the G-INS-i strategy following recommendations for sequences with global homology. ITS and ETS were aligned using the Q-INS-i strategy, which considers the secondary RNA structure (Katoh and Standley, 2013). The alignments were manually edited in Geneious 6.0 (Biomatters, 2015) to correct obvious alignment errors. In the absence of supported (Maximum Likelihood Bootstrap Support [MLBS] > 75%) phylogenetic incongruence between plastid and nuclear markers, the matrices were concatenated, also in Geneious.

Prior to molecular dating analysis, we performed Maximum Likelihood searches and compared our results with a previously published phylogeny of *Galeandra* (Monteiro et al., 2010, Gerlach & Pérez-Escobar, 2014) and Catasetinae (Pérez-Escobar et al., 2017b). Jmodeltest 2 (Darriba et al., 2012) was used to test the best model of DNA evolution giving the GTR + I + G model as the most suitable for our data. Maximum likelihood tree searches and bootstrapping of the combined dataset using 1000 replicates were performed in RAxML v. 8 (Stamatakis, 2006) using the graphical user interface raxmlGUI 1.3.1 (Silvestro and Michalak, 2012), under the GTR + G model of DNA evolution.

We subsequently time-calibrated our phylogeny, relying on the same matrix of four plastid and three nuclear markers, comprising 31 taxa and 6014 nucleotides, using a Bayesian relaxed-clock approach implemented in BEAST 1.8.3 (Drummond et al., 2012). Absolute divergence times were estimated under the GTR + G substitution model, and the Yule tree speciation model, which produced congruent absolute ages and Highest Posterior Densities (HDP) to those derived from Birth-Death tree models in Catasetinae (Pérez-Escobar et al., 2017c).

The Markov Chain Monte Carlo (MCMC) chain was run for 50 million generations, sampling every 10,000 generations. We performed three independent runs in BEAST, all of which derived virtually identical Maximum Clade Credibility (MCC) trees. Orchids appear to have diverged from the common ancestor of all other members of Asparagales in the Cretaceous around 110 Ma and the crown group ca. 90 Ma, and upper Epidendroids diverged in the Paleogene, around 50 Ma (Chomicki et al., 2015). There are four unambiguous Orchidaceae macrofossils, but none of them is assigned to taxa closely related to Catasetinae (Ramírez et al., 2007). Therefore, the phylogeny of *Galeandra* was secondarily calibrated based on the age obtained by Chomicki et al. (2015) for Catasetinae's crown group of 19.8 (95% Highest Posterior Density Interval [95% HPD]: 14.6–25.7 Ma). These ages are perfectly congruent with Givnish et al. (2015). A normal distribution prior was applied on the Most Recent Common Ancestor (MRCA) of Catasetinae, with Mean = 19.8 and Stdev = 3 (95% HPD 14.8–24.7). A maximum clade credibility tree was summarized in TreeAnnotator v. 1.8.0 (part of BEAST package) with a 25% burn-in, when effective sample sizes (ESS) for all parameters were superior to 200, as assessed in Tracer 1.5 (Rambaut et al., 2014). Trees were visualized and initially edited in FigTree 1.4.0 (Rambaut, 2009).

2.2. Ancestral area estimation

To estimate the probable geographic origin of *Galeandra* and where terrestrial species originated, we compiled geographic distribution data and performed ancestral area estimation analyses (AAE). Species ranges were coded from the literature and from herbarium specimens (ALCB, AMES, AMO, B, BM, BR, F, INPA, K, K-L, NY, P, PORT, RB, S, US and W - abbreviations according to Index Herbariorum (http://sweetgum.nybg.org/science/ih/). Biogeographical areas were derived from literature, as well as from distribution patterns observed in other plant lineages (e.g. Rubiaceae: Antonelli et al., 2009; Bromeliaceae: Givnish et al., 2011; *Cycnoches*: (Pérez-Escobar et al., 2017c). We coded the geographical range of *Galeandra* as: A = Central America, B = Chocó, C = Amazonia, D = Guiana Shield, E = Dry Diagonal and F = Atlantic Forest; Fig. S1 shows coded biogeographical regions. Specimens without reliable geographic locality or with dubious identification were excluded.

For ancestral area estimation, we relied on the R package BioGeoBEARS (Matzke, 2013), which evaluates several biogeographic models altogether to test for the contribution of evolutionary processes (i.e., range expansion, range extinctions, vicariance, founder-event speciation, within-area speciation) to explain the distribution of modern species. We analyzed independently three models: DEC (dispersal-extinction-cladogenesis) (Ree and Smith, 2008), which considers cladogenetic processes as the evolution of range at speciation events, estimating dispersal, extinction and range expansion by Maximum likelihood; a modified version of DIVA (dispersal-vicariance-analysis) (Ronquist, 1997), named DIVA-like, a method that allows dispersal and extinction in anagenetic processes and vicariance in cladogenetic processes; and a modified version of BayArea (Landis et al., 2013) or BayArea-like, a method designed to analyze a large number of areas. The founder-speciation parameter *j* was added separately, so that each model has been run with and without the *j* parameter. We assessed the overall fitness of the models conducting likelihood ratio tests based on AICc scores. *Galeandra macroplectra* was excluded from biogeographic analysis due to negative branch lengths

2.3. Range size

Terrestrial species usually tend to present larger distribution ranges, as observed by herbarium records. Therefore, we hypothesized that range size is associated to plant habit, and evaluated distribution ranges of all *Galeandra* species sampled in our phylogeny. Distribution ranges were obtained by literature and herbarium specimens' examination, as

stated in the previous section, totalizing 657 records. We calculated the Extent of Occurrence (EOO) and Area of Occupancy (AOO) for each species using GeoCAT (Bachman et al. 2011, http://geocat.kew.org). EOO represents the defined area contained within the shortest imaginary boundary drawn to encompass all the known sites of occurrence of a taxon, often measured by a minimum convex polygon; AOO represents the area within its "extent of occurrence" which is occupied by a taxon, usually calculated by the sum of all square grids in which the species were registered (IUCN, 2013). Because EOO extrapolates the area of occurrence of a species, we choose AOO for our analysis. The Table S5 shows the values of AOO measured and used in the analysis.

2.4. Ancestral state estimation of spur length

To investigate the role of trait shift in the floral spur length in *Galeandra,* we measured this trait in all species sampled in our phylogeny, and estimated its probable ancestral state and evolution through time. Fifteen out of the 20 know species of *Galeandra* (17 included in our DNA sequence matrix) were included in the analysis. We obtained minimum, mean and maximum values of spur length and width (Fig. S1) from herbarium specimens (the same described above for geographical records) and literature (Monteiro et al., 2010). Whenever possible, we gathered measures from at least five individuals per species. Table S6 provides a list with all measurements of species studied and herbarium specimens sourced. Maximum Likelihood Ancestral State Estimation (ASE) of mean spur length values was conducted using an ultrametric tree derived from dating analysis (see above) and the function *contMap* of the R package 'phytools' (Revell, 2012). This is Markov continuous-time model for continuous character and the most frequently used model for continuous character ancestral state estimations (Lewis 2001). In addition, to investigated the evolution of spur length through time, we produced a traitgram (Evans et al., 2009) by plotting our ultrametric tree as function of time (from root age to present) and phenotype (i.e. spur length) using the function *phenogram* of the package 'phytools'. Uncertainties of the ASEs were explored by plotting the probability density of the ancestral estimation in the traitgram.

2.5. Correlation tests

We further test for the correlated evolution between plant habit (0 = terrestrial, 1 = epiphytic) and spur length under a quantitative genetic threshold model (Wright, 1934; Felsenstein, 2012). This model is applied to discrete variables (e.g. viviparity: Lambert & Wiens, 2013; feeding mode in fishes: Revell, 2013), whose probability of state change is associated to an underlying continuous variable. Correlation analysis was implemented on a Bayesian framework for 1,000,000 generations, with a sampling fraction of 100 generations using the function *threshBayes* in the R package 'Phytools' (Revell, 2012).

2.6. Phylomorphospace

To visualize relationship between the spur length, range size and plant habit, while simultaneously accounting for phylogenetic relationship, we generated a morphospace using spur length and range size as continuous variables. To this end, we relied on the function *phylomorphospace* of the R package phytools (Revell, 2012).

2.7. Climatic variables

To investigate whether habitat and trait shift was correlated to changes in climatic niches, we sourced bioclimatic variables from georeferenced occurrences to characterize and compare the climatic niche of *Galeandra* species. We mapped 639 collection records obtained from floras, GBIF database and herbarium specimens (mean 37, maximum number of record per species 157). They represent the known

distribution of *Galeandra* and extant species included in our taxon sampling. To query GBIF database, we relied on the function *occ* of the R package SPOCC (Chamberlain, 2016). We extracted corresponding values of elevation and 19 climatic variables (30 s resolution) reflecting temperature and precipitation regimes from the WorldClim database (available at: http://www.worldclim.org/current; Hijmans et al., 2005), using the function *extract* of the R package RASTER (Hijmans, 2016).

2.8. Non-metric dimensional scaling analyses

To avoid spurious results of climatic niche modelling arising from inclusion of correlated variable, we determined the Pearson's correlation coefficients between the bioclim variables and altitude and then included only variables with a Pearson's correlation coefficient < 0.5, taking a single variable in correlated clusters. This way, we selected the bioclimatic variables 1 (Annual mean temperature), 2 (Mean diurnal temperature range), 12 (Annual precipitation), 13 (Precipitation of wettest week), 14 (Precipitation of driest week), and 18 (Precipitation of warmest quarter). We analyzed these variables using the R package VEGAN (Oksanen et al., 2007) to perform non-metric dimensional scaling analyses (NMDS) using the dataset of 657 georeferenced herbarium specimens. To ask whether (i) epiphytic versus terrestrial *Galeandra*, and (ii) short-spurred versus long-spurred *Galeandra* had different niches, we computed the 95% confidence intervals for each group. Overlap between confidence intervals suggests the absence of significant niche differentiation among groups.

3. Results

3.1. Phylogeny of Galeandra and time of origin of terrestrial and epiphytic clades

Our matrix of 31 taxa and 6014 nucleotides for four plastid and three nuclear genes yielded a tree recovering virtually the same relationships found by Monteiro et al., (2010) for *Galeandra* (Fig. S1, available at TreeBase under the accession number 22879). Our enlarged outgroup sampling scheme represents the genus level relationships in the tribe Catasetinae with high support for the core Catasetinae *sensu* (Dressler, 1983). In our phylogeny, *Galeandra* is sister group to the core Catasetinae (*Catasetum, Clowesia, Cycnoches, Mormodes, Dressleria*) plus *Grobya + Cyanaeorchis* (but larger datasets indicate *Grobya + Cynaeorchis* as sister to *Galeandra* + coreCatasetinae (Batista et al., 2014; Pérez-Escobar et al., 2017a, 2017b, 2016, 2015). *Galeandra devoniana* was recovered as sister to the remaining *Galeandra* species, which is split into a terrestrial and an epiphytic clade, both maximally supported (ML bootstrap support = 100). Absolute age estimation (Fig. 1, Fig. S2) yielded a root age (i.e. the split between *Eulophia* and *Cyrtopodium* + Catasetina) of 29 Ma (95% HPD: 17–44). The MRCA of *Galeandra* plus the core Catasetinae age is 17 Ma (95% HPD = 11–23), and the crown group of *Galeandra* originated in the Miocene ca. 9 Ma (95% HPD: 5–13). The terrestrial and epiphytic clades diverged from each other in the late Miocene about 7 Ma (95% HPD: 4–11) (Fig. 1). The epiphyte clade included species from Amazonia, Mexico, Venezuela, and Guianas, while the terrestrial clade encompassed species occurring in the Dry Diagonal of South America, e.g. Cerrado, and open environments in Colombia and Venezuela. The terrestrial and epiphytic clades crown ages are approximately 4 Ma (95% HPD: 2–6). Most of the *Galeandra* diversification occurred in the Pliocene-Pleistocene border (> 3 Ma). The Mexican clade of epiphytes diverged 2 Ma (95% HPD: 1–5).

3.2. Geographic origin of terrestrial habit and biogeographic history of Galeandra

BioGeoBEARS multi-model approach yielded DEC + j as the best fitting model for the *Galeandra* phylogeny (Tab. S3 provides AICc values of all biogeographical models tested). The MRCA of *Galeandra* is inferred to have lived in an area encompassing Amazonia and Guiana Shield (Fig. 1). The ancestral area for the terrestrial clade, which has at least one widespread species, *G. beyrichii*, included Amazonia, Dry Diagonal and Atlantic Forest. On the other hand, the MRCA of the epiphytic clade is restricted to Northern South America, i.e. Amazonia and Guiana Shield. Among the epiphytic *Galeandra,* only *G. blanchetii* occurs in (and is restricted to) open vegetation of the Dry Diagonal.

3.3. Terrestrial habit origin and correlates with spur length evolution, range size and climatic niche

Terrestrial and epiphytic species revealed marked differences in spur length, as shown by the continuous trait map of floral spur evolution in *Galeandra* (Fig. 2). Short spur length was the ancestral condition in *Galeandra* flowers (Fig. 2A), being the shortest in *G. devoniana*. The terrestrial clade shows an intermediate pattern of floral spur length. *Galeandra beyrichi* presents the shortest spur in this clade. The MRCA of the epiphytic clade had longer floral spurs, and *G. magnicolumna* is the species with the largest floral spurs. The traitgram revealed contrasting morphological rate changes of spur length (Fig. 2B; and density traitgram in Fig. S4) between most epiphyte and terrestrial species. We observed a shift in morphological rates at the base of the long-spurred clade of *Galeandra* (epiphytic species) (Fig. 2B). Terrestrial and short-spurred clade presents a higher rate of morphological change.

Changes in habit, range sizes and floral spur length were only correlated in epiphytic species. They formed a cluster of lineages with narrow range sizes (up to 200 km^2) and longer floral spur (2–2.5 cm) (Fig. 3). Only epiphytic *Galeandra devoniana* did not integrate with this group. Instead, it occupied a different space between clusters of taxa with short spur length and moderately small range size. The terrestrial species did not form a cohesive group, and they did not present a clear pattern of range size or floral spur length.

The NDMS analysis found only the bioclimatic variables 1, 2, 12, 13, 14 and 18 to be non-correlated (Fig. S5). No niche differentiation was detected between terrestrial and epiphytic *Galeandra*, nor between long and short-spurred *Galeandra* (Fig. S6 and Fig. S7).

4. Discussion

4.1. Miocene origin of Galeandra in Amazonia

Galeandra arose towards the Late Miocene, about 10 Ma, close to the climatic optimum when several plant clades in the Neotropical Region originated (Antonelli et al., 2009; Gustafsson et al., 2010; Hoorn et al., 2010; Pérez-Escobar et al., 2017c, Schey et al. 2018). At that time, the Guiana and the Brazilian Shields became large islands. A period of intensified mountain uplift started at the same time in Northern, promoting the origin and extinction of mega wetlands in Amazonia, and shifting the drainage of the Amazon Basin eastwards (Antonelli et al., 2009; Hoorn et al., 2010).

Our ancestral area estimation analyses suggest the MRCA of *Galeandra* inhabited Amazonia (including the Guiana Shield) towards the Late Miocene, at a period where the region occupied a very large part of South America, extending as far as South Parana region (Hoorn et al., 2010). Interchange of lineages between Amazonia – the primary source of Neotropical biodiversity - and Cerrado, and Chaco increased considerably during Miocene (Antonelli et al. 2018). Early diversification of *Galeandra* occurred in forested areas, while diversification of terrestrial lineages in *Galeandra* might have taken place in both forested areas and the Dry Diagonal, influenced by the rise of dry vegetation biomes. The extent of tropical forests in South America changed with the Andean uplift and together with global climate change, favored the establishment of the Dry vegetation areas in South America (Antonelli et al., 2009; Posadas and Ortiz-Jaureguizar, 2016).
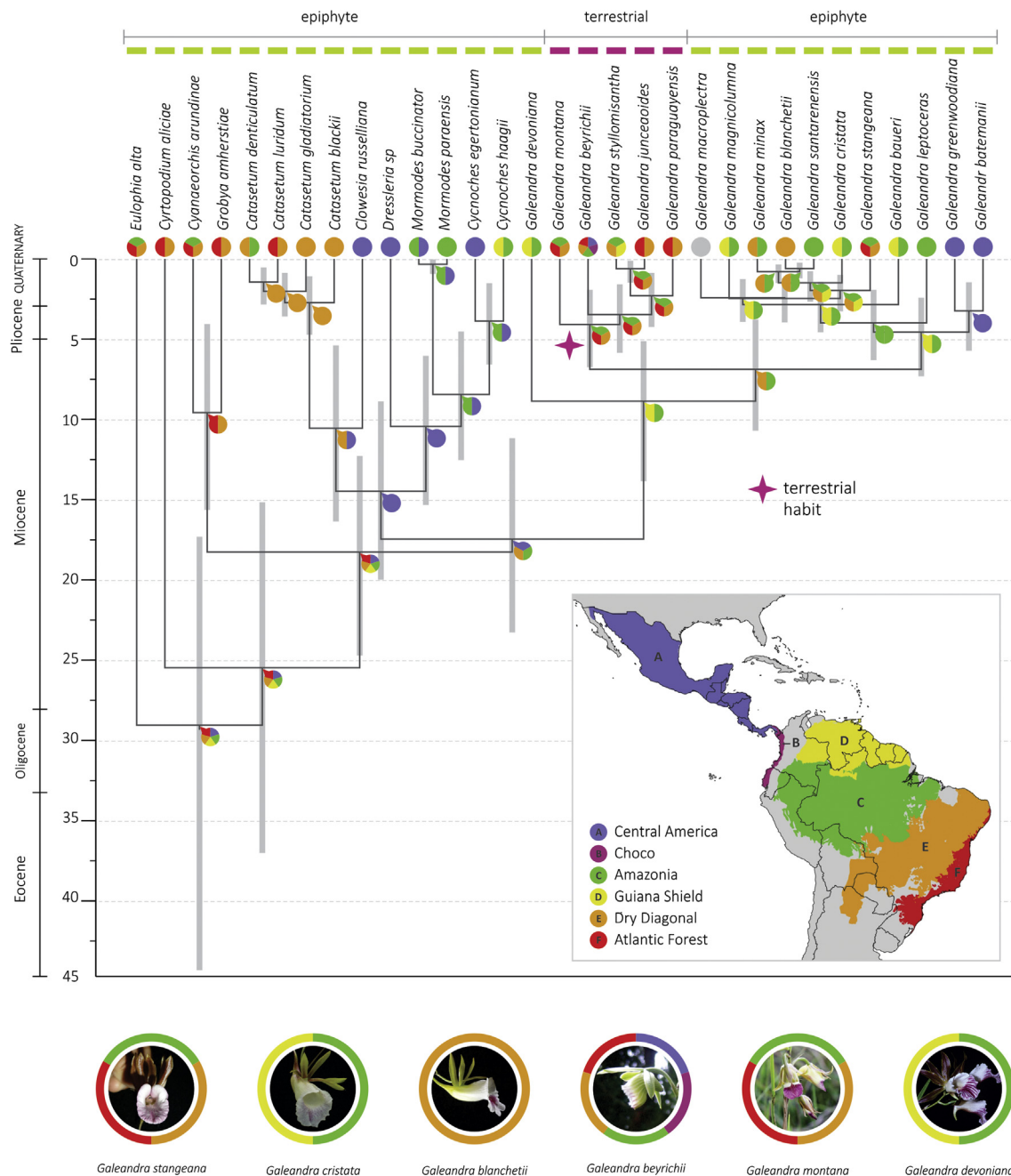
Fig. 1. Time-calibrated phylogeny of *Galeandra* and ancestral area estimations, showing area coding, and distribution map. Pie charts on nodes represent ancestral areas derived from the BioGeoBEARS analysis and color code follows the legend on the map: A = Central America, B = Choco, C = Amazonia, D = Guiana Shield, E = Dry Diagonal and F = Atlantic Forest. Grey bars represent 95% HDP interval for absolute ages. Colored circles on tips represent the occurrence of that species in the delimited geographical areas. *Galeandra macroplectra* presents a grey circle, because it was excluded from the BioGeoBEARS analysis because of negative branch lengths. Photographs in circles represent floral morphological variation in the genus. Colors encircling orchid pictures represent the distribution range of the species according to delimited areas on the map. Photo credits: Adarilda Petini-Benelli (*G. stangeana*, *G. blanchetii*), Günter Gerlach (*G. devoniana*), Silvana H. N. Monteiro (*G. cristata*, *G. montana*).

The establishment of a complex system of dry vegetation biomes, including the Cerrado and old world savannas by the end of Pliocene started in Miocene, influenced by aridification and C4 grasses expansion and increasing fire activity (Simon et al., 2009; Hoetzel et al., 2013; Pennington and Hughes, 2014). Such dry areas are mostly found in the dry diagonal of South America, but with accessory patches occurring in all Neotropical Region (Werneck, 2011). In the case of Cerrado, the boundaries appear to have been porous to the migration and recruitment of lineages from a range of wet and dry forest vegetation types (Werneck, 2011).

Adjacent biomes including the Amazonia and Atlantic wet forests, tropical and subtropical dry thorn scrub (Caatinga and Chaco), subtropical grasslands, and wetlands have all contributed to the recruitment of Cerrado lineages (Machado et al., 2018; Sobral-Souza et al., 2015). The migration of lineages from surrounding ecosystems was facilitated by its nested distribution and enhanced the striking Cerrado's species richness (Machado et al., 2018; Simon et al., 2009). The MRCA of the terrestrial *Galeandra* (4 Ma: HDP 2–6) could have originated on the tropical forests (Fig. 3) or on the recently formed open vegetation areas. In spite of beginning the diversification about 10 Ma, most plant
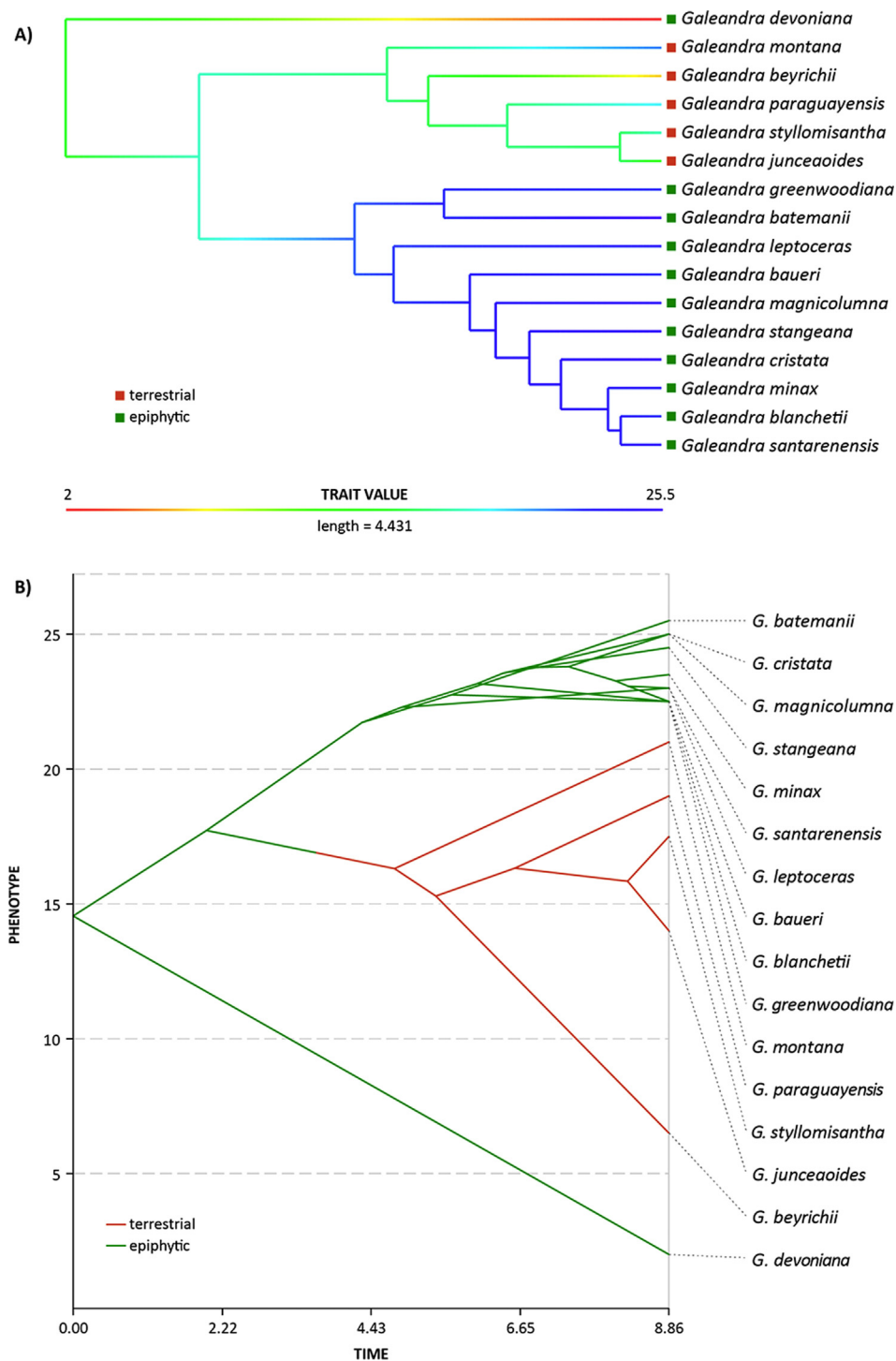
Fig. 2. Spur length evolution in *Galeandra* species. A. Ancestral state estimation of spur length in *Galeandra* species, showing short spur length as de ancestral state Color pattern indicates spur length according to the scale; B. Phenogram of spur length evolution in time, contrasting morphological rate changes of spur length between most epiphyte and terrestrial species.

lineages characteristic of Cerrado diversified only recently, 4 Ma or less (Roncal et al., 2013; Simon et al., 2009). Therefore, the diversification time of both clades (epiphytes and terrestrial) is coincident with this period of drastic transformation in the distribution of forest and open vegetation. However, present day distribution suggests that only terrestrial *Galeandra* species were able to occupy the recently formed dryer biomes, while expanding their distribution. Terrestrial *Galeandra* species occupy several different open vegetation areas, not only the core Cerrado vegetation, but also costal dunes in Brazil, savannas of

Venezuela, islands of savanna in Amazon. An exception to this pattern is *G. beyrichii*, which is adapted to shaded humid forests (Monteiro et al., 2010).

Tropical broadleaf forests provide a plethora of niches for epiphytic plants, and both origins of epiphytism and these forests are profoundly connected (Schuettpelz and Pryer, 2009). Therefore, it is not surprising that the early diversification of the epiphytic clade of *Galeandra* occurred on the forested areas (Amazonia and Guiana Shield) until the Pliocene, and in the Dry Diagonal in the Quaternary only (Fig. 3). These
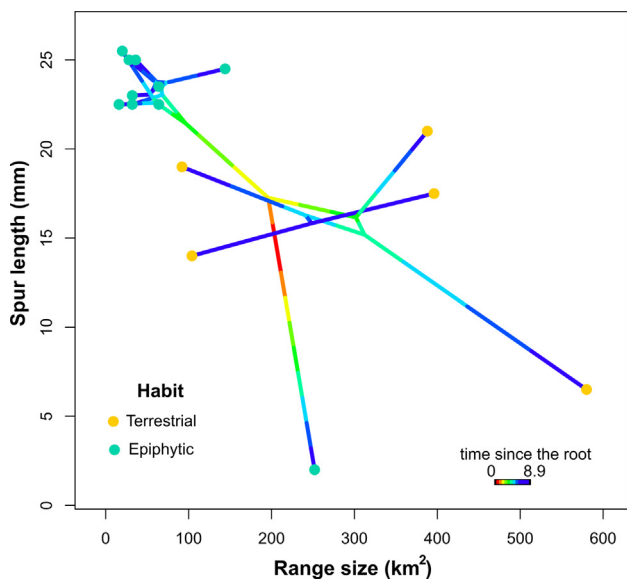
Fig. 3. Phylomorphospace analysis result for *Galeandra* species, considering simultaneously spur length and range size as continuous variables, and phylogenetic relationships. Each dot in the graphics represent one *Galeandra* species and the colours distinguish them by habit, i.e. terrestrial (yellow) and epiphytic (green). *X* and *Y* axes represent respectively, range size (in km^2) and floral spur length (in cm). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

epiphytes do not occupy the open vegetation, but the gallery forests, which are riverine forests forming narrow strips along the river valleys in the Cerrado biome (Oliveira-Filho and Ratter, 1995). Many plants and animal species from Amazonian or Atlantic Forest domains crosses the Cerrado through those gallery forests, some expanding their distribution within Cerrado (Costa, 2003). The affinities between the woody floras of these ecosystems have been pointed out by several authors e.g. (Gottsberger and Silberbauer-Gottsberger, 2006; Oliveira-Filho and Fontes, 2000). For the particular case of *Galeandra*, the gallery forests are occupied by Amazonian species, reinforcing the relatedness of Amazon and gallery forests (Oliveira-Filho and Ratter, 1995; Pennington et al., 2006).

4.2. Habit, floral spur length, range size and climatic niche

Changes from epiphytic to terrestrial habitat might have played a role in *Galeandra* ecological requirements far beyond light and water levels and may have also affected interactions with bee pollinators. Epiphytic species presents longer floral spurs, which can be associated to the evolution with long tongued pollinators, such as Euglossini bees, the primary pollinators of Catasetinae orchids (Dressler, 1982; Ramírez et al., 2011).

Pollination observations in *Galeandra* flowers are scarce, limited to some punctual observations of pollinaria attached to male orchid bee's body (i.e. fragrance seeking) (Pearson and Dressler, 1985; Romero-Gonzalez and Warford, 1995) or "Anthophoridae" bees (possibly *Xylocopa*) (nectar or pollen seeking) (Chase and Hills, 1992; Romero-Gonzalez and Warford, 1995). However, how *Galeandra* attract their pollinators remains a mystery. Floral spurs in epiphytic *Galeandra* apparently lack any nutritional reward to pollinators (G. Gerlach, Munich Bot. Gard. pers. comm.), indicating a possible deceptive attraction, very common in orchids (Ackerman, 1986; Jersáková et al., 2006; Nilsson, 1998; Pansarin and Maciel, 2017). Fragrance-seeking bees can find rewards at least in *G. devoniana*, *G. magnicolumna* and *G. stangeana* (SHNM pers. obs.) Observations on cultivated epiphytic species of *Galeandra (G. cristata, G. santarenensis, G. stangeana)* shows that they are self-compatible, but not able to self-pollinate, therefore requiring cross-

pollination (SHNM pers. obs.).

Terrestrial species have a variable spur length, but in general shorter than epiphytic. The occupation of terrestrial habits was possibly accompanied by a shift in pollinator's guild or independence of pollination by animals. Euglossini bees are diverse and widespread in forested habitats, mostly on cloud or lowland forests (Cameron, 2004; Dressler, 1982), presenting low diversity in open vegetation habitats like Cerrado (Faria and Silveira, 2011). The orchid bee fauna occurring in open vegetation biomes are frequently associated to patches of forests occurring along the rivers and there is no species endemic to these biomes, but shared with adjacent large forested biomes like Amazon or Atlantic Forest (Faria and Silveira, 2011). Because flowers of terrestrial *Galeandra* appear to be rewardless (G. Gerlach pers. comm. to OAPE in June 2016), pollination by deceit might also occur in this clade. Also, terrestrial *G. beyrichii* and *G. montana* present very wide distribution ranges and high levels of fruit production in herbaria material, suggesting self-pollination is common among these taxa. However, evidences for self-compatibility, but not spontaneous self-pollination, were observed on cultivated plants of the terrestrial *G. styllomisantha*.

The distribution of orchids might be limited by the joint effect of habitat availability, seed dispersal restrictions and pollination limitation (Gravendeel et al., 2004; Winkler et al. 2009; McCormick and Jacquemyn, 2014). Epiphytic *Galeandra* usually presents a narrow geographic range size compared to the terrestrial species. It might be linked to the habit occupied *per se* or to different mechanisms of pollination (dependent of pollinators or not). It could also reflect a higher dependency on particular pollinators. Smaller range sizes are typically found in more specialized mutualisms, as compared to their generalist relatives (Chomicki et al., 2015b). Some evidences of epiphytic orchids with a restricted distribution when compared to the geographic range size of terrestrial species were indicated by Zhang et al. (2015) which related this difference to environmental variables. The low availability of substrates in epiphytic habitats results in restricted and irregular moisture supplies, making water shortages a limiting factor for the establishment and growth of epiphytes (Benzing, 1987; Laube and Zotz, 2003; Zhang et al., 2015).

Terrestrial species do not form a cohesive group regarding range size or floral spur length. Slow morphological change in interaction-related traits is a feature of highly specialized mutualisms, and suggests stabilizing selection (Davis et al., 2014; Chomicki and Renner, 2017). Our morphological analysis shows terrestrial *Galeandra* has a high spur morphate (Fig. 2), potentially resulting from disruption of bee pollination. The lack of cohesiveness of terrestrial species's traits probably indicate a relaxed selection in habit and spur length.

5. Conclusions

Galeandra, a primarily epiphytic orchid lineage, arose in Miocene about 10 Ma in South America, and most probably the epiphytic clade diversified in the Amazon. Terrestrial habit in *Galeandra* arose synchronously with the expansion of open vegetation savannas around 5 Ma. Surprisingly the shift from epiphytic to terrestrial habitat does not involve significant changes in climatic niche, partly explaining, together with higher competition in terrestrial habits, the low frequency of such transition in Epidendroid orchids. However, terrestrial species tend to occupy larger geographical ranges probably facilitated by their ecological requirements, but also pollination mode. Floral morphology suggests a shift from pollination by long tongued bees to pollination by morphologically distinct bees or independence from animal pollination. Our study highlights how climate change driving major vegetation changes correlates with habit evolution, and how habit evolution impacts other aspects of Neotropical plant biology, here mutualistic interactions with pollinators.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ympev.2018.06.041.

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